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(FILE 'HOME' ENTERED AT 17:21:27 ON 26 OCT 2004)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, ...' ENTERED AT 17:22:45 ON 26 OCT 2004
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189 FILE AGRICOLA
90 FILE ANABSTR
5 FILE ANTE
8 FILE AQUALINE
19 FILE AQUASCI
91 FILE BIOBUSINESS
5 FILE BIOCOMMERCE
124 FILE BIOENG
710 FILE BIOSIS
148 FILE BIOTECHABS
148 FILE BIOTECHDS
123 FILE BIOTECHNO
567 FILE CABA
12 FILE CANCERLIT
1612 FILE CAPLUS
48 FILE CEABA-VTB
2 FILE CEN
5 FILE CIN
2 FILE CONFSCI
36 FILE CROPB
75 FILE CROPU
36 FILE DDFB
49 FILE DDFU
107 FILE DGENE
39 FILE DISSABS
36 FILE DRUGB
66 FILE DRUGU
7 FILE EMBAL
208 FILE EMBASE
223 FILE ESBIODASE
7* FILE FEDRIP
2 FILE FOREGE
181 FILE FROSTI
329 FILE FSTA
30 FILE GENBANK
4 FILE HEALSAFE
215 FILE IFIPAT
63 FILE JICST-EPLUS
8 FILE KOSMET
192 FILE LIFESCI
184 FILE MEDLINE
6 FILE NIOSHTIC
9 FILE NTIS
3 FILE OCEAN
347 FILE PASCAL
1 FILE PHIN
101 FILE PROMT
1 FILE RDISCLOSURE
489 FILE SCISEARCH
1 FILE SYNTHLINE
320 FILE TOXCENTER
1917 FILE USPATFULL
144 FILE USPAT2
3 FILE VETU
18 FILE WATER
210 FILE WPIDS
3 FILE WPIFV
210 FILE WPINDEX
15 FILE IPA
9 FILE NAPRALERT
8 FILE NLDB

L1 QUE (EUGENO? OR (FERUL?(S) ACID?))(S)(CONIFERY? OR VANILL?)

FILE 'USPATFULL, CAPLUS, BIOSIS, CABA, SCISEARCH, PASCAL, FSTA,
TOXCENTER, ESBIOBASE, IFIPAT, WPIDS, EMBASE, LIFESCI, AGRICOLA, MEDLINE'
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L2 7712 S (EUGENO? OR (FERUL?(S)ACID?))(S)(CONIFERY? OR VANILL?)
L3 210 S L2(S)(DEHYDROGENAS? OR SYNTHAS? OR SYNTHETAS? OR KETOTHIOLAS
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=> index bioscience medicine

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48 FILE CEABA-VTB
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5 FILE CIN
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36 FILE DDFB
49 FILE DDFU
107 FILE DGENE

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=> d rank
F1      1917  USPATFULL
F2      1612  CAPLUS
F3       710  BIOSIS
F4       567  CABA
F5       489  SCISEARCH
F6       347  PASCAL
F7       329  FSTA
F8       320  TOXCENTER
F9       223  ESBIODBASE
F10      215  IFIPAT
F11      210  WPIDS
F12      210  WPINDEX
F13      208  EMBASE
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F16      184  MEDLINE
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F34       36  DDFB
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F44        8  KOSMET
F45        8  NLDB
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F52        4  HEALSAFE
F53        3  OCEAN
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OR DEMETHYLAS?)

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PROCESSING COMPLETED FOR L4
L5 9 DUP REM L4 (28 DUPLICATES REMOVED)

=> d ti l5

L5 ANSWER 1 OF 9 IFIPAT COPYRIGHT 2004 IFI on STN
TI PRODUCTION OF P-HYDROXYBENZOIC ACID

=> d ti 15 2-9

L5 ANSWER 2 OF 9 IFIPAT COPYRIGHT 2004 IFI on STN
TI PRODUCTION OF VANILLIN; REACTING TRANS-FERULIC ACID AND COENZYME A
(COASH) UNDER TRANS-FERULATE:COASH LIGASE ENZYME ACTIVITY, TRANS-FERULOYL
SCOA HYDRATASE ACTIVITY, AND 4-HYDROXY-3-METHOXYPHENYL-BETA-
HYDROXYPROPIONYL SCOA CLEAVAGE ACTIVITY; PSEUDOMONAS ENZYMES

L5 ANSWER 3 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on
STN DUPLICATE 1
TI Functional analyses of genes involved in the metabolism of ferulic acid in
Pseudomonas putida KT2440

L5 ANSWER 4 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on
STN DUPLICATE 2
TI Cloning and characterization of the ferulic acid catabolic genes of
Sphingomonas paucimobilis SYK-6

L5 ANSWER 5 OF 9 IFIPAT COPYRIGHT 2004 IFI on STN
TI PRODUCTION OF VANILLIN; REACTING TRANS-FERULIC ACID AND COENZYME A
(COASH) UNDER TRANS-FERULATE:COASH LIGASE ENZYME ACTIVITY, TRANS-FERULOYL
SCOA HYDRATASE ACTIVITY, AND 4-HYDROXY-3-METHOXYPHENYL-BETA-
HYDROXYPROPIONYL SCOA CLEAVAGE ACTIVITY; PSEUDOMONAS ENZYMES

L5 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
TI Organisms with inactivated enzymes of eugenol and/or ferulic acid
catabolism and their use for production of substituted phenols

L5 ANSWER 7 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on
STN DUPLICATE 3
TI Bioconversion of ferulic acid into vanillic acid by means of a
vanillate-negative mutant of Pseudomonas fluorescens strain BF13

L5 ANSWER 8 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on
STN DUPLICATE 4
TI Biochemical and genetic analyses of ferulic acid catabolism in Pseudomonas
sp strain HR199

L5 ANSWER 9 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on
STN DUPLICATE 5
TI Biotransformation of eugenol to vanillin by a mutant of Pseudomonas sp
strain HR199 constructed by disruption of the vanillin dehydrogenase (vdh)
gene

=> d ibib abs 15 1-9

L5 ANSWER 1 OF 9 IFIPAT COPYRIGHT 2004 IFI on STN
AN 10423087 IFIPAT;IFIUDB;IFICDB
TITLE: PRODUCTION OF P-HYDROXYBENZOIC ACID
INVENTOR(S): Gasson; Michael John, Norfolk, GB
Narbad; Arjan, Norfolk, GB
Rhodes; Michael John Charles, Norfolk, GB
Walton; Nicholas John, Norfolk, GB
PATENT ASSIGNEE(S): Unassigned
AGENT: Michael L. Goldman NIXON PEABODY LLP, Clinton Square,
P.O. Box 31051, Rochester, NY, 14603-1051, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2003167511	A1	20030904
APPLICATION INFORMATION:	US 2002-199405		20020717

APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
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Section 371 PCT Filing OF:WO 1997-GB809 19970324 UNKNOWN
 DIVISION OF: US 1998-155185 19980922
 DIVISION OF: US 2000-733383 20001207

	NUMBER	DATE
PRIORITY APPLN. INFO.:	GB 1996-6187	19960323
FAMILY INFORMATION:	US 2003167511	20030904
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	Patent Application - First Publication	
	CHEMICAL	
	APPLICATION	

NUMBER OF CLAIMS: 86 19 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1 describes the **vanillin** pathway in *Pseudomonas fluorescens* biovar. V, strain AN103. HMPHP SCoA is 4-hydroxy-3-methoxyphenyl-beta-hydroxypropionyl SCoA. I is an enzyme that catalyses the interconversion of trans-**ferulic acid** and transferuloyl SCoA; II is an enzyme that catalyses the interconversion of trans-**feruloyl** SCoA and HMPHP SCoA; III is an enzyme that catalyses the interconversion of HMPHP SCoA and **vanillin**; and IV is an enzyme that catalyses the interconversion of **vanillin** and **vanillic acid**.

FIG. 2 illustrates the growth of strain AN103 following transfer to MM medium containing 10 mM **vanillate** (V), 10 mM transferulate (F) or 10 mM trans-**ferulate** plus 10 mM **vanillate** (FV). Cultures were previously grown in MM medium containing 10 mM **vanillate**.

FIG. 3 indicates the changes in trans-**ferulate** and **vanillate** concentrations during growth of strain AN 103 on MM medium containing 10 mM trans-ferulate.

FIG. 4 shows the production of **vanillin** (van) and **vanillate** (VA) by an extract of cells of strain AN103 (165 mu g protein) incubated with trans-**ferulate**, ATP, CoASH and Mg²⁺ ions, both in the absence of NAD⁺ and in its presence (0.5 mM). Cells were grown in the presence of 10 mM trans-**ferulate**, plus 10 mM **vanillate**.

FIG. 5 demonstrates the formation of **feruloyl** SCoA, **vanillin** and acetyl SCoA from trans-**ferulate** supplied to a PD10-treated extract of trans-**ferulate**-grown cells of strain AN103 (7 mu g protein) in the presence of ATP, CoASH and Mg²⁺ ions.

FIG. 6 demonstrates the production of **vanillin**, acetyl SCoA and **feruloyl** SCoA from HMPHP SCoA supplied to a PD10-treated cellfree extract (7 mu g protein) of trans-**ferulate**-grown cells of strain AN103.

FIG. 7 shows the induction over time of trans-**ferulate**:CoASH ligase activity in response to 10 mM trans-**ferulate** (F), 10 mM **vanillate** (V) and 10 mM trans-**ferulate** plus 10 mM **vanillate** (FV) present in MM medium. The inocula were grown in MM medium plus 10 mM **vanillate**; growth conditions, enzyme extraction and assay were as described in Examples 1 and 2.

FIG. 8 shows SDS-PAGE of A), an extract of cells grown in MM medium with 10 mM trans-**ferulate**, electrophoresed at successive stages of purification of the HMPHP SCoA cleavage enzyme; successive stages are Crude Extract, Mono Q-purified, Mono-Ppurified and Phenyl Superose-purified, and B), extracts of cells grown in MM medium with either 10 mM **vanillate** or 10 mM trans-**ferulate** and electrophoresed alongside Mono-P-purified cleavage enzyme; A) silver-stained; B) Coomassie-stained.

FIG. 9 shows EcoRI/PstI digests of cosmid clones pFI793, pFI794, pFI795 and pFI796 separated on an agarose gel.

FIG. 10 shows the sequence of the redundant primers designed from 20 N-terminal amino residues of the 31-kDa protein (SEQ ID Nos. 5 and 6).

FIG. 11 shows a Southern blot of EcoRI/PstI digests of various cosmid clones probed with the PCR product amplified using the Nterminal degenerate oligonucleotide primers as shown in FIG. 10.

FIG. 12 shows the nucleotide sequence of pFI989 (ie the 4370 bp EcoRI/PstI fragment from pFI794), together with the succeeding 882 bp determined from a further subclone, pFI1056 and from pFI794 itself (SEQ ID No 7). The amino acid sequence of the 31 kD protein and that corresponding to the succeeding open reading frame encoding **vanillin**:NAD⁺ oxidoreductase (**vanillin** dehydrogenase) (SEQ ID Nos. 2 and 4) are also shown.

FIG. 13 shows the nucleotide sequence of pFI901 (ie the 1.8 kb EcoRI/PstI fragment from pFI793) (SEQ ID No 8).

FIG. 14 shows the nucleotide sequence of pFI911 (ie the 850 bp EcoRI/PstI fragment from pFI793) (SEQ ID No 9).
 FIG. 15 shows the nucleotide sequence of pFI912 (ie the 958 bp EcoRI/PstI fragment from pFI793) (SEQ ID No 10).
 FIG. 16 shows the nucleotide sequence of pFI913 (ie the 959 bp EcoRI/PstI fragment from pFI793) (SEQ ID No 11).
 FIG. 17 is a diagrammatic representation of the outward reading primers for pFI901 (P35 and P39), pFI911 (P32 and P36), pFI912 (P33 and P37) and pFI913 (P34 and P38).
 FIG. 18 is a diagrammatic representation showing the formation of the 1.5 kb PCR product, using primers P34 and P39, which spans the region in the cosmid between the **inserts** of pFI913 and pFI901.
 FIG. 19 shows the nucleotide sequence of the merged contigs pFI913/PCR product/pFI901 (4259 bp) (SEQ ID No 12).

AB One aspect of the present invention relates to a transgenic plant which, by presence of a transgene, is able to produce phydroxybenzoic acid or a beta -D-glycoside or beta -D-glucose ester thereof. A method is also disclosed for producing phydroxybenzoic acid or a beta -D-glycoside or beta -D-glucose ester thereof using a transgenic plant of the present invention.

CLMN 86 19 Figure(s).

FIG. 1 describes the **vanillin** pathway in *Pseudomonas fluorescens* biovar. V, strain AN103. HMPHP SCoA is 4-hydroxy-3-methoxyphenyl-beta-hydroxypropionyl SCoA. I is an enzyme that catalyses the interconversion of trans-**ferulic acid** and transferuloyl SCoA; II is an enzyme that catalyses the interconversion of trans-**feruloyl** SCoA and HMPHP SCoA; III is an enzyme that catalyses the interconversion of HMPHP SCoA and **vanillin**; and IV is an enzyme that catalyses the interconversion of **vanillin** and **vanillic acid**.

FIG. 2 illustrates the growth of strain AN103 following transfer to MM medium containing 10 mM **vanillate** (V), 10 mM transferulate (F) or 10 mM trans-**ferulate** plus 10 mM **vanillate** (FV). Cultures were previously grown in MM medium containing 10 mM **vanillate**.

FIG. 3 indicates the changes in trans-**ferulate** and **vanillate** concentrations during growth of strain AN 103 on MM medium containing 10 mM trans-ferulate.

FIG. 4 shows the production of **vanillin** (van) and **vanillate** (VA) by an extract of cells of strain AN103 (165 mu g protein) incubated with trans-**ferulate**, ATP, CoASH and Mg²⁺ ions, both in the absence of NAD⁺ and in its presence (0.5 mM). Cells were grown in the presence of 10 mM trans-**ferulate**, plus 10 mM **vanillate**.

FIG. 5 demonstrates the formation of **feruloyl** SCoA, **vanillin** and acetyl SCoA from trans-**ferulate** supplied to a PD10-treated extract of trans-**ferulate**-grown cells of strain AN103 (7 mu g protein) in the presence of ATP, CoASH and Mg²⁺ ions.

FIG. 6 demonstrates the production of **vanillin**, acetyl SCoA and **feruloyl** SCoA from HMPHP SCoA supplied to a PD10-treated cellfree extract (7 mu g protein) of trans-**ferulate**-grown cells of strain AN103.

FIG. 7 shows the induction over time of trans-**ferulate**:CoASH ligase activity in response to 10 mM trans-**ferulate** (F), 10 mM **vanillate** (V) and 10 mM trans-**ferulate** plus 10 mM **vanillate** (FV) present in MM medium. The inocula were grown in MM medium plus 10 mM **vanillate**; growth conditions, enzyme extraction and assay were as described in Examples 1 and 2.

FIG. 8 shows SDS-PAGE of A), an extract of cells grown in MM medium with 10 mM trans-**ferulate**, electrophoresed at successive stages of purification of the HMPHP SCoA cleavage enzyme; successive stages are Crude Extract, Mono Q-purified, Mono-Ppurified and Phenyl Superose-purified, and B), extracts of cells grown in MM medium with either 10 mM **vanillate** or 10 mM trans-**ferulate** and electrophoresed alongside Mono-P-purified cleavage enzyme; A) silver-stained; B) Coomassie-stained.

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FIG. 14 shows the nucleotide sequence of pFI911 (ie the 850 bp EcoRI/PstI fragment from pFI793) (SEQ ID No 9).

FIG. 15 shows the nucleotide sequence of pFI912 (ie the 958 bp EcoRI/PstI fragment from pFI793) (SEQ ID No 10).

FIG. 16 shows the nucleotide sequence of pFI913 (ie the 959 bp EcoRI/PstI fragment from pFI793) (SEQ ID No 11).

FIG. 17 is a diagrammatic representation of the outward reading primers for pFI901 (P35 and P39), pFI911 (P32 and P36), pFI912 (P33 and P37) and pFI913 (P34 and P38).

FIG. 18 is a diagrammatic representation showing the formation of the 1.5 kb PCR product, using primers P34 and P39, which spans the region in the cosmid between the **inserts** of pFI913 and pFI901.

FIG. 19 shows the nucleotide sequence of the merged contigs pFI913/PCR product/pFI901 (4259 bp) (SEQ ID No 12).

L5 ANSWER 2 OF 9 IFIPAT COPYRIGHT 2004 IFI on STN
 AN 03987942 IFIPAT;IFIUDB;IFICDB
 TITLE: PRODUCTION OF VANILLIN; REACTING TRANS-FERULIC ACID
 AND COENZYME A (COASH) UNDER TRANS-FERULATE:COASH
 LIGASE ENZYME ACTIVITY, TRANS-FERULOYL SCOA HYDRATASE
 ACTIVITY, AND 4-HYDROXY-3-METHOXYPHENYL-BETA-
 HYDROXYPROPIONYL SCOA CLEAVAGE ACTIVITY; PSEUDOMONAS
 ENZYMES
 INVENTOR(S): Gasson; Michael John, Norfolk, GB
 Narbad; Arjan, Norfolk, GB
 Rhodes; Michael John Charles, Norfolk, GB
 Walton; Nicholas John, Norfolk, GB
 PATENT ASSIGNEE(S): Plant Bioscience Limited, Norwich, GB
 PRIMARY EXAMINER: Saidha, Tekchand
 AGENT: Nixon Peabody LLP

	NUMBER	PK	DATE
PATENT INFORMATION:	US 6664088	B2	20031216
APPLICATION INFORMATION:	US 2001014467	A1	20010816
EXPIRATION DATE:	US 2000-733383		20001207
	3 May 2019		

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DOCUMENT TYPE:	US 2001014467	20010816
FILE SEGMENT:	Utility	
	Granted Patent - Utility, with Pre-Grant Publication	
	CHEMICAL	
	GRANTED	

PARENT CASE DATA:

This application is a divisional of U.S. patent application Ser. No. 09/155,183 (now U.S. Pat. No. 6,323,011 B1), which was filed on May 3, 1999 (and accepted May 3, 1999) under 35 U.S.C. section 371 as a national stage application of

PCT/GB97/00809 filed Mar. 24, 1997, claiming priority of Great Britain Application No. 9606187.4 filed Mar. 23, 1996. The biological material listed below has been deposited under the Budapest Treaty at The National Collections of Industrial and Marine Bacteria Limited (23 St. Machar Drive, Aberdeen AB2 1RY, Scotland, UK):

**** TABLE ****

NCIMB No. Description Date of Deposit 40783 *Pseudomonas fluorescens* biovar V (strain Jan. 15, 1996 AN103) 40777 *Escherichia coli* (strain pFI793) containing Dec. 15, 1995 cosmid pFI703

NOTE: INDEXED FROM APPLICATION
NUMBER OF CLAIMS: 16
GRAPHICS INFORMATION: 27 Drawing Sheet(s), 27 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1 describes the **vanillin** pathway in *Pseudomonas fluorescens* biovar. V, strain AN103. HMPHP SCoA is 4-hydroxy-3-methoxyphenyl-beta-hydroxypropionyl SCoA. I is an enzyme that catalyses the interconversion of trans-**ferulic acid** and transferuloyl SCoA; II is an enzyme that catalyses the interconversion of trans-**feruloyl** SCoA and HMPHP SCoA; III is an enzyme that catalyses the interconversion of HMPHP SCoA and ***vanillin***; and IV is an enzyme that catalyses the interconversion of ***vanillin*** and **vanillic acid**.

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FIG. 3 indicates the changes in trans-**ferulate** and **vanillate** concentrations during growth of strain AN 103 on MM medium containing 10 mM trans-**ferulate**.

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vanillin dehydrogenase) (SEQ ID Nos. 2 and 4) are also shown.
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FIG. 19 shows the nucleotide sequence of the merged contigs pFI913/PCR product/pFI901 (4259 bp) (SEQ ID No 12).

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NTE INDEXED FROM APPLICATION

CLMN 16

GI 27 Drawing Sheet(s), 27 Figure(s).

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L5 ANSWER 3 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on
STN DUPLICATE 1

ACCESSION NUMBER: 2003:571208 SCISEARCH

THE GENUINE ARTICLE: 695ZY

TITLE: Functional analyses of genes involved in the metabolism of
ferulic acid in *Pseudomonas putida* KT2440

AUTHOR: Plaggenborg R; Overhage J; Steinbuchel A; Priefert H
(Reprint)

CORPORATE SOURCE: Univ Munster, Inst Mikrobiol, Corrensstr 3, D-48149
Munster, Germany (Reprint); Univ Munster, Inst Mikrobiol,
D-48149 Munster, Germany

COUNTRY OF AUTHOR: Germany

SOURCE: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (JUN 2003) Vol.
61, No. 5-6, pp. 528-535.
Publisher: SPRINGER-VERLAG, 175 FIFTH AVE, NEW YORK, NY
10010 USA.
ISSN: 0175-7598.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 36

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB *Pseudomonas putida* KT2440 is a physiologically extremely versatile non-pathogenic bacterium that is applied as a "biosafety strain" in biotechnological processes, as authorized by the USA National Institute of Health. Analysis of the *P. putida* KT2440 whole-genome sequence revealed the genetic organization of the genes *fcs*, *ech*, and *vdh*, which are essential for **ferulic acid** conversion to **vanillic acid** via **vanillin**. To confirm the physiological function of these structural genes as **feruloyl-CoA synthetase** (*Fcs*), **enoyl-CoA hydratase/aldolase** (*Ech*), and **vanillin dehydrogenase** (*Vdh*), respectively, they were cloned and expressed in *Escherichia coli*. Recombinant strains harboring *fcs* and *ech* were able to transform **ferulic acid** to **vanillin**. The enzyme activities of *Fcs* and *Vdh* were determined in protein extracts of these cells. The essential involvement of *fcs*, *ech* and *vdh* in the catabolism of **ferulic acid** in *P. putida*

KT2440 was proven by separately **inactivating** each gene by **insertion** of Omega-elements. The corresponding mutant strains KT2440fcsOmegaKm, KT2440echOmegaKm, and KT2440vdhOmegaKm were not able to grow on **ferulic acid**. The potential application of *P. putida* KT2440 and the mutant strains in biotechnological **vanillin** production process is discussed.

L5 ANSWER 4 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on
STN DUPLICATE 2

ACCESSION NUMBER: 2002:737818 SCISEARCH
THE GENUINE ARTICLE: 588TX
TITLE: Cloning and characterization of the ferulic acid catabolic genes of *Sphingomonas paucimobilis* SYK-6
AUTHOR: Masai E (Reprint); Harada Y; Peng X
CORPORATE SOURCE: Nagaoka Univ Technol, Dept Bioengn, Nagaoka, Niigata 9402188, Japan (Reprint); Tokyo Univ Agr & Technol, Grad Sch Bioapplicat & Syst Engn, Koganei, Tokyo 1848588, Japan
COUNTRY OF AUTHOR: Japan
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (SEP 2002) Vol. 68, No. 9, pp. 4416-4424.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
ISSN: 0099-2240.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 36

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB *Sphingomonas paucimobilis* SYK-6 degrades **ferulic acid** to **vanillin**, and it is further metabolized through the protocatechuate 4,5-cleavage pathway. We obtained a TnS mutant of SYK-6, FA2, which was able to grow on **vanillic acid** but not on **ferulic acid**. A cosmid which complemented the growth deficiency of FA2 on **ferulic acid** was isolated. The 5.2-kb BamHI-EcoRI fragment in this cosmid conferred the transformation activity of **ferulic acid** to **vanillin** on *Escherichia coli* host cells. A sequencing analysis revealed the genes *ferB* and *ferA* in this fragment; these genes consist of 852- and 2,127-by open reading frames, respectively. The deduced amino acid sequence of *ferB* showed 40 to 48% identity with that of the **feruloyl-coenzyme A (CoA) hydratase/lyase** genes of *Pseudomonas* and *Amycolatopsis ferulic acid* degraders. On the other hand, the deduced amino acid sequence of *ferA* showed no significant similarity to the **feruloyl-CoA synthetase** genes of other **ferulic acid** degraders. However, the deduced amino acid sequence of *ferA* did show 31% identity with **pimeloyl-CoA synthetase** of *Pseudomonas mendocina* 35, which has been classified as a new superfamily of **acyl-CoA synthetase** (ADP forming) with **succinyl-CoA synthetase** (L. B. Sanchez, M. Y. Galperin, and M. Muller, *J. Biol. Chem.* 275: 5794-5803, 2000). On the basis of the enzyme activity of *E. coli* carrying each of these genes, *ferA* and *ferB* were shown to encode a **feruloyl-CoA synthetase** and **feruloyl-CoA hydratase/lyase**, respectively. **p-coumaric acid**, **caffeic acid**, and **sinapinic acid** were converted to their corresponding benzaldehyde derivatives by the cell extract containing *FerA* and *FerB*, thereby indicating their broad substrate specificities. We found a *ferB* homolog, *ferB2*, upstream of a 5-carboxyvanillic acid decarboxylase gene (*ligW*) involved in the degradation of 5,5'-dehydrodivanillic acid. The deduced amino acid sequence of *ferB2* showed 49% identity with *ferB*, and its gene product showed **feruloyl-CoA hydratase/lyase** activity with a substrate specificity similar to that of *FerB*. **Insertional inactivation** of each *fer* gene in *S. paucimobilis* SYK-6 suggested that the *ferA* gene is essential and that *ferB* and *ferB2* genes are involved in **ferulic acid** degradation.

L5 ANSWER 5 OF 9 IFIPAT COPYRIGHT 2004 IFI on STN
AN 10014465 IFIPAT;IFIUDB;IFICDB
TITLE: PRODUCTION OF VANILLIN; REACTING TRANS-FERULIC ACID AND COENZYME A (COASH) UNDER TRANS-FERULATE:COASH LIGASE ENZYME ACTIVITY, TRANS-FERULOYL SCOA HYDRATASE ACTIVITY, AND 4-HYDROXY-3-METHOXYPHENYL-BETA-

HYDROXYPROPIONYL SCOA CLEAVAGE ACTIVITY; PSEUDOMONAS ENZYMES

INVENTOR(S): Gasson; Michael John, Norfolk, GB
 Narbad; Arjan, Norfolk, GB
 Rhodes; Michael John Charles, Norfolk, GB
 Walton; Nicholas John, Norfolk, GB

PATENT ASSIGNEE(S): Unassigned

PATENT ASSIGNEE PROBABLE: Plant Bioscience Ltd GB (Probable)

AGENT: Michael L. Goldman NIXON PEABODY LLP, Clinton Square,
 P.O. Box 31051, Rochester, NY, 14603, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2001014467	A1	20010816
APPLICATION INFORMATION:	US 2000-733383		20001207

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
Section 371 PCT Filing OF:	WO 1997-GB809	19970324	UNKNOWN
DIVISION OF:	US 1999-155183	19990503	

	NUMBER	DATE
PRIORITY APPLN. INFO.:	GB 1996-61874	19960323
FAMILY INFORMATION:	US 2001014467	20010816
	US 6664088	20031216
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	Patent Application - First Publication	
	CHEMICAL APPLICATION	

NUMBER OF CLAIMS: 66 19 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1 describes the **vanillin** pathway in *Pseudomonas fluorescens* biovar. V, strain AN103. HMPHP SCoA is 4-hydroxy-3-methoxyphenyl-beta-hydroxypropionyl SCoA. I is an enzyme that catalyses the interconversion of trans-**ferulic acid** and transferuloyl SCoA; II is an enzyme that catalyses the interconversion of trans-**feruloyl** SCoA and HMPHP SCoA; III is an enzyme that catalyses the interconversion of HMPHP SCoA and ***vanillin***; and IV is an enzyme that catalyses the interconversion of ***vanillin*** and **vanillic acid**.

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CLMN 66 19 Figure(s).

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L5 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2004 ACS on STN
 ACCESSION NUMBER: 2000:289140 CAPLUS
 DOCUMENT NUMBER: 132:319715
 TITLE: Organisms with inactivated enzymes of eugenol and/or ferulic acid catabolism and their use for production of substituted phenols
 INVENTOR(S): Rabenhorst, Juergen; Steinbuechel, Alexander; Priefert, Horst; Overhage, Joerg
 PATENT ASSIGNEE(S): Haarmann & Reimer G.M.b.H., Germany
 SOURCE: Ger. Offen., 80 pp.
 CODEN: GWXXBX
 DOCUMENT TYPE: Patent
 LANGUAGE: German
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
DE 19850242	A1	20000504	DE 1998-19850242	19981031
WO 2000026355	A2	20000511	WO 1999-EP7952	19991020
WO 2000026355	A3	20001109		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU,

CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL,
 IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD,
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 RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE,
 DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF,
 CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
 BR 9914930 A 20010710 BR 1999-14930 19991020
 EP 1124947 A2 20010822 EP 1999-953892 19991020
 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,
 IE, SI, LT, LV, FI, RO
 AU 761093 B2 20030529 AU 2000-10413 19991020
 JP 2003533166 T2 20031111 JP 2000-579727 19991020
 DE 1998-19850242 A 19981031
 WO 1999-EP7952 W 19991020
 PRIORITY APPLN. INFO.:

AB The invention concerns a transformed and/or a mutagenized uni- or multi-cellular organism, which is characterized by the fact that enzymes of the eugenol and/or ferulic acid catabolism are inactivated such that an accumulation of the intermediate coniferyl alc., coniferyl aldehyde, ferulic acid, vanillin, and/or vanillic acid takes place. Thus, *Pseudomonas* with inactivating insertions or deletions in the *vdh*, or *vat* and *aat*, genes were produced and used in prodn. of vanillin, ferulic acid, and coniferyl alc.

L5 ANSWER 7 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN DUPLICATE 3

ACCESSION NUMBER: 2000:424499 SCISEARCH
 THE GENUINE ARTICLE: 319TP
 TITLE: Bioconversion of ferulic acid into vanillic acid by means of a vanillate-negative mutant of *Pseudomonas fluorescens* strain BF13
 AUTHOR: Civolani C; Barghini P; Roncetti A R; Ruzzi M (Reprint); Schiesser A
 CORPORATE SOURCE: UNIV TUSCIA, DIPARTIMENTO AGROBIOL & AGROCHIM, VIA C LELLIS BLOCCO B, I-01100 VITERBO, ITALY (Reprint); UNIV TUSCIA, DIPARTIMENTO AGROBIOL & AGROCHIM, I-01100 VITERBO, ITALY
 COUNTRY OF AUTHOR: ITALY
 SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (JUN 2000) Vol. 66, No. 6, pp. 2311-2317.
 Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904.
 ISSN: 0099-2240.
 DOCUMENT TYPE: Article; Journal
 FILE SEGMENT: LIFE; AGRI
 LANGUAGE: English
 REFERENCE COUNT: 35

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB From a **ferulic-acid**-degrading *Pseudomonas fluorescens* strain (BF13), we have isolated a transposon mutant, which retained the ability to bioconvert **ferulic acid** into **vanillic acid** but lost the ability to further degrade the latter **acid**. The mutant, BF13-97, was very stable, and therefore it was suitable to be used as a biocatalyst for the preparative synthesis of **vanillic acid** from **ferulic acid**. By use of resting cells we determined the effect on the bioconversion rate of several parameters, such as the addition of nutritional factors, the concentration of the biomass, and the carbon source on which the biomass was grown. The optimal yield of **vanillic acid** was obtained with cells pregrown on M9 medium containing p-coumaric **acid** (0.1% [wt/vol]) as a sole carbon source and yeast extract (0.001% [wt/vol]) as a source of nutritional factors. Under these conditions, 1 mg (wet weight) of biomass produced 0.23 mg of **vanillic acid** per h. The genomic region of BF13-97 flanking the transposon's site of **insertion** was cloned and sequenced revealing two open reading frames of 1,062 (*varA*) and 954 (*vanB*) bp, respectively. The *van* genes are organized in a cluster and encode the subunits of the **vanillate-O-demethylase**, which catalyzes the first step of the **vanillate** catabolism, Amino **acid** sequences deduced from *vanA* and *vanB* genes were shown

to have high identity with known VanAs and VanBs from *Pseudomonas* and *Acinetobacter* spp, Highly conserved regions known to exist in class IA oxygenases were also found in the **vanillate-O-demethylase** components from *P. fluorescens* BF13, The terminal oxygenase VanA is characterized by a conserved Rieske-type [2Fe-2S] (R) ligand center, The reductase VanB contains a plant-type ferredoxin [2Fe-2S] (Fd), flavin mononucleotide, and NAD-ribose binding domains which are located in its C-terminal and N-terminal halves, respectively. Transfer of wild-type vanAB genes to BF13-97 complemented this mutant, which recovered its ability to grow on either **vanillic** or **ferulic acid**.

L5 ANSWER 8 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on
STN DUPLICATE 4

ACCESSION NUMBER: 1999:854619 SCISEARCH
THE GENUINE ARTICLE: 252AQ
TITLE: Biochemical and genetic analyses of ferulic acid catabolism in *Pseudomonas* sp strain HR199
AUTHOR: Overhage J; Priefert H (Reprint); Steinbuchel A
CORPORATE SOURCE: UNIV MUNSTER, INST MIKROBIOL, CORRENSSTR 3, D-48149 MUNSTER, GERMANY (Reprint); UNIV MUNSTER, INST MIKROBIOL, D-48149 MUNSTER, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (NOV 1999) Vol. 65, No. 11, pp. 4837-4847.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0099-2240.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 48

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The gene loci *fcs*, encoding **feruloyl** coenzyme A (**feruloyl-CoA**) **synthetase**, *ech*, encoding enoyl-CoA hydratase/aldolase, and *aat*, encoding beta-ketothiolase, which are involved in the catabolism of **ferulic acid** and **eugenol** in *Pseudomonas* sp, strain HR199 (DSM7063), were localized on a DNA region covered by two EcoRI fragments (E230 and E94), which were recently cloned from a *Pseudomonas* sp, strain HR199 genomic library in the cosmid pVK100, The nucleotide sequences of parts of fragments E230 and E94 were determined, revealing the arrangement of the aforementioned genes. To confirm the function of the structural genes *fcs* and *ech*, they were cloned and expressed in *Escherichia coli*, Recombinant strains harboring both genes were able to transform **ferulic acid** to **vanillin**, The **feruloyl-CoA synthetase** and enoyl-CoA hydratase/aldolase activities of the *fcs* and *ech* gene products, respectively, were confirmed by photometric assays and by high-pressure liquid chromatography analysis. To prove the essential involvement of the *fcs*, *ech*, and *aat* genes in the catabolism of **ferulic acid** and **eugenol** in *Pseudomonas* sp, strain HR199, these genes were **inactivated** separately by the **insertion** of omega elements. The corresponding mutants *Pseudomonas* sp, strain HR*fcs* Omega Gm and *Pseudomonas* sp, strain HR*ech* Omega Km were not able to grow on **ferulic acid** or on **eugenol**, whereas the mutant *Pseudomonas* sp, strain HR*aat* Omega Km exhibited a **ferulic acid-** and **eugenol-**positive phenotype like the wild type, In conclusion, the degradation pathway of **eugenol** via **ferulic acid** and the necessity of the activation of **ferulic acid** to the corresponding CoA ester was confirmed. The *aat* gene product was shown not to be involved in this catabolism, thus excluding a beta-oxidation analogous degradation pathway for **ferulic acid**. Moreover, the function of the *ech* gene product as an enoyl-CoA hydratase/aldolase suggests that **ferulic acid** degradation in *Pseudomonas* sp. strain HR199 proceeds via a similar pathway to that recently described for *Pseudomonas fluorescens* AN103.

L5 ANSWER 9 OF 9 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on
STN DUPLICATE 5
ACCESSION NUMBER: 1999:943195 SCISEARCH

THE GENUINE ARTICLE: 261KJ
 TITLE: Biotransformation of eugenol to vanillin by a mutant of Pseudomonas sp strain HR199 constructed by disruption of the vanillin dehydrogenase (vdh) gene
 AUTHOR: Overhage J; Priefert H (Reprint); Rabenhorst J; Steinbuchel A
 CORPORATE SOURCE: UNIV MUNSTER, INST MIKROBIOL, D-48149 MUNSTER, GERMANY (Reprint); UNIV MUNSTER, INST MIKROBIOL, D-48149 MUNSTER, GERMANY; HAARMANN & REIMER GMBH, D-37601 HOLZMINDEN, GERMANY
 COUNTRY OF AUTHOR: GERMANY
 SOURCE: APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (NOV 1999) Vol. 52, No. 6, pp. 820-828.
 Publisher: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010.
 ISSN: 0175-7598.
 DOCUMENT TYPE: Article; Journal
 FILE SEGMENT: LIFE; AGRI
 LANGUAGE: English
 REFERENCE COUNT: 34

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The catabolism of **eugenol** in Pseudomonas sp. strain HR199 (DSM7063) proceeds via **coniferyl** alcohol **coniferyl** aldehyde, **ferulic acid**, **vanillin**, **vanillate** and protocatechuate, which is further degraded by the ortho-cleavage pathway. The **vanillin dehydrogenase** of Pseudomonas sp. strain HR199, which catalyses the NAD (+) dependent oxidation of **vanillin** to **vanillate**, was **inactivated** by the **insertion** of omega elements into the vdh gene, which was characterized recently. Omega elements conferring resistance against kanamycin (Omega Km) or gentamycin (Omega Gm) were constructed by polymerase chain reaction amplification of the aminoglycoside 3'-O-phosphotransferase gene and the gentamycin-3-acetyltransferase gene, using the plasmids pSUP5011 and pBBR1MCS-5 respectively as template DNA. A 211-bp BssHII fragment of the vdh gene was substituted by Omega Km or nGm, and the functional vdh gene was replaced by vdh Omega Km or vdh Omega Gm in Pseudomonas sp. strain HR199 by homologous recombination. Cells of the mutant Pseudomonas sp, strain HRvdh Omega Km, pregrown on gluconate, accumulated up to 2.9 mM **vanillin** during incubation in mineral medium with 6.5 mM **eugenol**. As a result of another **vanillin dehydrogenase** activity (VDH-II), the accumulated **vanillin** was further degraded, when **coniferyl** aldehyde was exhausted from the medium. Characterization of the purified VDH-II revealed the identity of this enzyme with the recently characterized **coniferyl**-aldehyde **dehydrogenase**

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(FILE 'HOME' ENTERED AT 17:21:27 ON 26 OCT 2004)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, ...' ENTERED AT 17:22:45 ON 26 OCT 2004
 SEA (EUGENO? OR (FERUL?(S)ACID?)) (S) (CONIFERY? OR VANILL?)

 189 FILE AGRICOLA
 90 FILE ANABSTR
 5 FILE ANTE
 8 FILE AQUALINE
 19 FILE AQUASCI
 91 FILE BIOBUSINESS
 5 FILE BIOCOMMERCE
 124 FILE BIOENG
 710 FILE BIOSIS
 148 FILE BIOTECHABS
 148 FILE BIOTECHDS
 123 FILE BIOTECHNO
 567 FILE CABA

12 FILE CANCERLIT
 1612 FILE CAPLUS
 48 FILE CEABA-VTB
 2 FILE CEN
 5 FILE CIN
 2 FILE CONFSCI
 36 FILE CROPB
 75 FILE CROPU
 36 FILE DDFB
 49 FILE DDFU
 107 FILE DGENE
 39 FILE DISSABS
 36 FILE DRUGB
 66 FILE DRUGU
 7 FILE EMBAL
 208 FILE EMBASE
 223 FILE ESBIODBASE
 7* FILE FEDRIP
 2 FILE FOREGE
 181 FILE FROSTI
 329 FILE FSTA
 30 FILE GENBANK
 4 FILE HEALSAFE
 215 FILE IFIPAT
 63 FILE JICST-EPLUS
 8 FILE KOSMET
 192 FILE LIFESCI
 184 FILE MEDLINE
 6 FILE NIOSHTIC
 9 FILE NTIS
 3 FILE OCEAN
 347 FILE PASCAL
 1 FILE PHIN
 101 FILE PROMT
 1 FILE RDISCLOSURE
 489 FILE SCISEARCH
 1 FILE SYNTHLINE
 320 FILE TOXCENTER
 1917 FILE USPATFULL
 144 FILE USPAT2
 3 FILE VETU
 18 FILE WATER
 210 FILE WPIDS
 3 FILE WPIFV
 210 FILE WPINDEX
 15 FILE IPA
 9 FILE NAPRALERT
 8 FILE NLDB

L1 QUE (EUGENO? OR (FERUL?(S) ACID?))(S)(CONIFERY? OR VANILL?)

FILE 'USPATFULL, CAPLUS, BIOSIS, CABA, SCISEARCH, PASCAL, FSTA,
 TOXCENTER, ESBIODBASE, IFIPAT, WPIDS, EMBASE, LIFESCI, AGRICOLA, MEDLINE'
 ENTERED AT 17:25:57 ON 26 OCT 2004

L2 7712 S (EUGENO? OR (FERUL?(S) ACID?))(S)(CONIFERY? OR VANILL?)
 L3 210 S L2(S)(DEHYDROGENAS? OR SYNTHAS? OR SYNTHETAS? OR KETOTHIOLAS
 L4 37 S L3 (S)(INACTIVA? OR DELET? OR INSERT?)
 L5 9 DUP REM L4 (28 DUPLICATES REMOVED)

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